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CYTOECOLOGICAL STUDIES OF SILENE ROTUNDIFOLIA NUTT., S. VIRGINICA L., AND HYBRID[1]

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This paper is the result of certain cytoecological studies of *Silene rotundifolia* Nutt, the Round-leafed catchfly, and *S. virginica* L., the Fire pink (CARYOPHYLLACEAE). *S. rotundifolia* was described by Nuttall (6) in 1807. Its habitat was described as "moist ledges of rock cliffs" in Ohio and Tennessee. Since then its distribution has been more accurately mapped from herbarium records and it is known to occur on rock ledges or in cliff crevices of limestone or sandstone throughout its range in Ohio, Tennessee, Kentucky, Virginia, West Virginia, and northern Alabama and Georgia. These habitats which are exposed to high light intensities often appear dry, but these plants are neither "xerophytes," nor is water usually a limiting factor, as the tap root of *S. rotundifolia* extends into a water source in the joint and bedding planes. Wolfe, Wareham, and Scofield (7) in their work on microclimates in southern Ohio show that the environmental conditions in rock crevices are considerably different from those in the surrounding forest and open areas.

S. virginica, which was studied in conjunction with *S. rotundifolia*, has a much wider distribution. It grows in open woodlands and forest borders in southern New Jersey, western New York and southwestern Ontario to Minnesota, and south to Missouri and Georgia (1, 4, 5). Although these two species often grow in the same region, one has never been known to invade the habitat of the other, nor has hybridization of the two been known to occur in the field.

In order to further analyze the ecological requisites of this species, hybrid material for study was obtained by crossing *S. rotundifolia* with *S. virginica*, one of the two other red flowered species of *Silene* growing in Ohio. Crosses with *S. rotundifolia* as the ovule parent and reciprocal crosses were made and repeated successfully. These species are cross fertile and approximately 100 seeds externally resembling those of the ovule parent develop in each ovulary after cross fertilization.

The F_1 hybrid, like both parents, is perennial, slender, ascending or reclining, viscid-pubescent, branched; the blades of the lower leaves taper into winged petioles, the upper leaves are sessile; the calyx is tubular-campanulate, somewhat enlarged by the ripening pod. The lower and basal leaves of the hybrid are intermediate between the obovate or broadly spatulate leaf blades of *S. rotundifolia* and the oblanceolate leaf blades of *S. virginica*. The upper leaves are intermediate between the broadly oblong or orbicular-ovate leaves of *S. rotundifolia* and oblong-lanceolate leaves of *S. virginica*. The petals of the hybrid flowers are intermediate between those of the parent species in size, color and shape. The petals are two-cleft or two-lobed as are those of both parents, but the degree of laciniation varies between the deeply incised two-cleft petals of *S. rotundifolia* and the less incised two-cleft petals of *S. virginica*.

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The number of flowers per plant and the length of the flowering period is greater in the hybrid than in either parent. The vegetative growth of the hybrid is luxuriant and many perfect flowers develop on each plant. The amount of pollen in each anther of the hybrid is much less than that of either parent, and the filaments are often weak and short. In both parent species, the petals open, five stamens elongate, the anthers begin to dehisce, and then the other five stamens and the styles elongate or the filaments elongate. This is followed by elongation of the styles and expansion of the three stigmas. Pollination usually takes place as the pistils grow to their ultimate height. However, the stamens of the hybrid sometimes never elongate, or they do not dehisce until after the pistils have grown above them, or the filaments collapse before the anthers dehisce. Thus self-pollination often fails to occur in the hybrid, but this failure was eliminated in experimentation by hand pollinating. After pollination occurs in the hybrid, the ovulary begins to swell, finally dehiscing when mature; but only shrunken, shriveled ovules occur within the small capsule.

From the following breeding results it appears that the hybrid is both self and cross sterile:

S. hybrid ♀	× (repeated 1500 times) no seeds	*S. hybrid* ♂
S. hybrid ♀	× (repeated 131 times) no seeds	*S. rotundifolia* ♂
S. hybrid ♀	× (repeated 103 times) no seeds	*S. virginica* ♂

There are usually more than 100 ovules in each ovulary and 1,734 hybrid flowers were either self or cross pollinated. None of the 170,000 ovules observed developed into seeds.

This failure of viable seed to develop does not appear to be the result of adverse environmental conditions as *S. rotundifolia* and *S. virginica* plants growing at the same time in conditions similar to those of the hybrid were "setting" seed in the ovularies of both cross and self-pollinated flowers. Cytological studies were then undertaken in an attempt to explain the failure of seeds to develop.

Observations of microsporogenesis were made in *S. rotundifolia*, *S. virginica*, and the hybrid.

Slides were prepared for studying microsporogenesis by smearing the anthers of one bud in acetocarmine. The anthers in the buds collected in early morning contained more pollen mother cells in desirable stages than those collected later in the day. Immediately after collection, the buds were put in a 3 : 1 absolute alcohol-acetic acid fixing solution. Twenty-four hours later they were transferred to a 70% alcohol solution.

S. rotundifolia pollen mother cells observed had regular meiotic divisions with the formation of four microspores. Cells observed in late diakinesis and early metaphase I contained 24 bivalents (Fig. 1 A), and those in anaphase I had 24 univalents moving toward each pole (Fig. 1 B), illustrating regular pairing and disjunction. The diploid chromosome number of *S. rotundifolia* is 48.

Microsporogenesis is also regular in *S. virginica*. Although no counts were made from cells in metaphase I, pairing was regular and all bivalents lined up on the metaphase plate as in Fig. 1 C. All cells observed in metaphase II had 24 univalents in each nucleus, (Fig. 1 D). Pairing and disjunction appear to be regular. The diploid chromosome number of *S. virginica* is 48.

Blackburn (3) reported the diploid chromosome number of 44 species of *Silene* in various sections of Europe as 24. In 1928 she reported *S. schafta* to have a diploid chromosome number of 24, 48, or 192. Love (3) in 1942 found *S. pontica* (Rumania) and *S. vallesia* (Alps) to have diploid chromosome numbers of 48.

No records of chromosome counts on American species of *Silene* were found reported in the literature. Relative to the European species, these American species appear to be basically tetraploid.

Many meiotic irregularities occur during microsporogenesis in the hybrid. Chromosome studies revealed that this hybrid has a diploid number of 48, as do both *S. rotundifolia* and *S. virginica*, and that gross morphology of all its 48 chromosomes is so much alike that it is not possible to identify those of maternal or paternal origin (Figs. 2 A-B). Pairing was highly irregular; univalents, bivalents, and

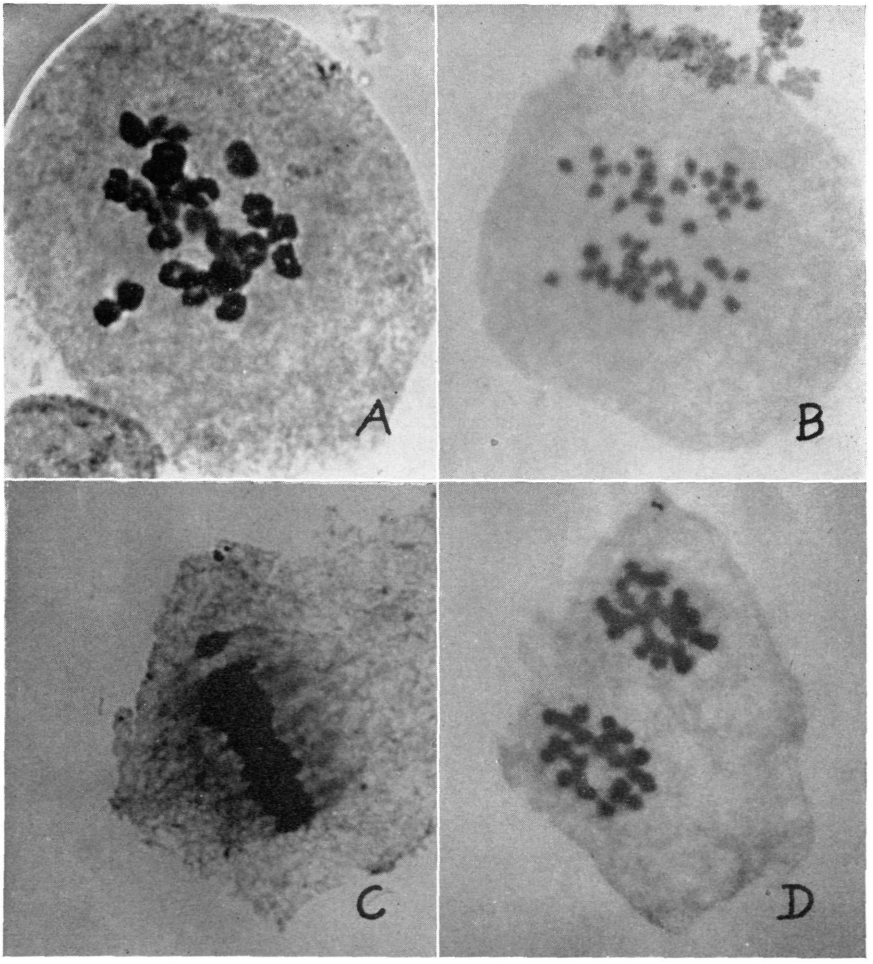

FIG. 1. (A) *Silene rotundifolia*: late diakinesis, 24 bivalents. (B) *S. rotundifolia*: anaphase I, 24 univalents moving toward each pole. Note the similar morphology of all chromosomes. (C) *S. virginica*: metaphase I. (D) *S. virginica*: metaphase II, 24 univalents in each nucleus. 1000×. Photomicrographs by Tillman J. Johnson.

multiple associations being formed in varying numbers from one cell to another (Fig. 2 B). Lagging was often observed in both the first and second meiotic divisions (Fig. 2 C). The lagging chromosomes moved at random to one pole or the other or they were sometimes incorporated in micronuclei. Although four microspores were usually formed from each microspore mother cell, the number varied from one to six (Fig. 2 D). Each of these irregularities, failure of synapsis, formation of multiple associations, lagging of chromosomes, unequal

numbers of chromosomes moving to each pole, and the formation of micronuclei, would contribute toward disturbance of the genetic balance of gametes and therefore to the sterility of the hybrid.

Although *S. rotundifolia* grows on cliff faces and rock ledges and *S. virginica* grows in open woodlands and woodland borders, they are often geographically

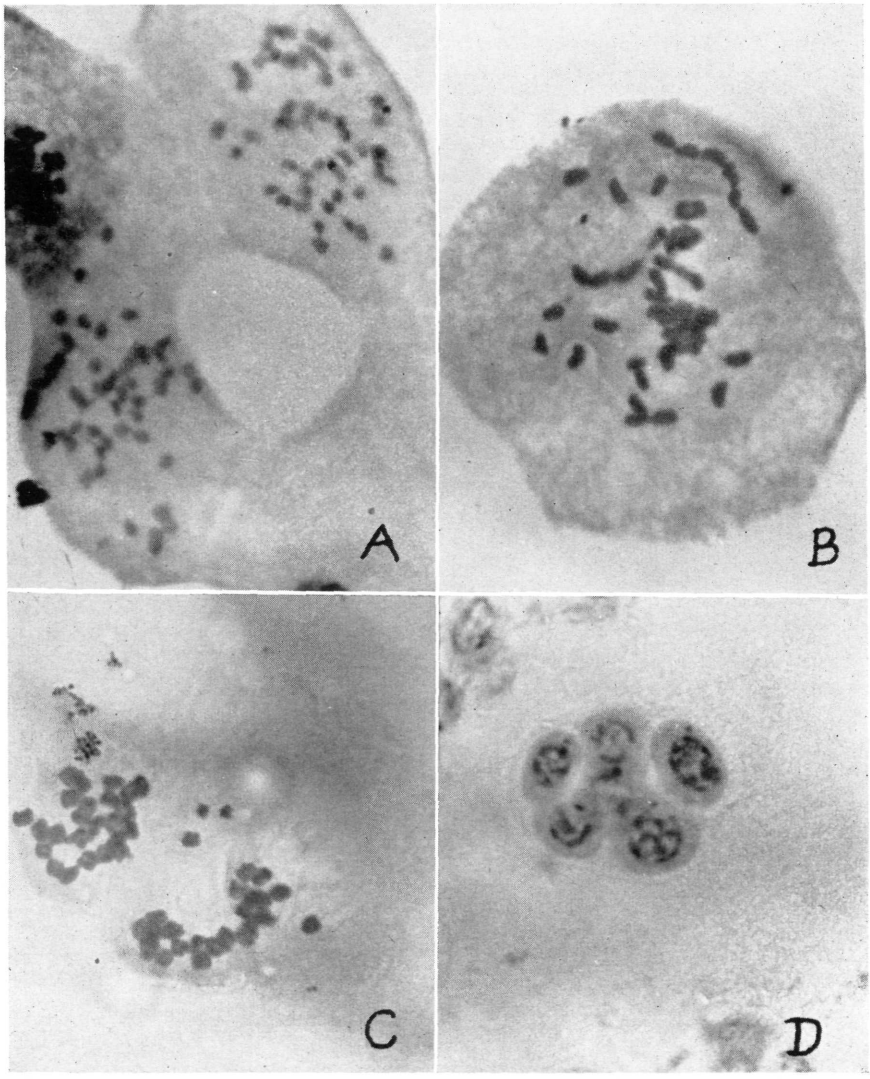

FIG. 2. (A) Hybrid: metaphase I, pairing irregular, chromosomes of each genome morphologically similar. (B) Hybrid: metaphase I, univalents, bivalents, and multiple associations present. (C) Hybrid: anaphase I, three chromosomes lagging. (D) Hybrid: polyspory (more than four spores) resulting from meiotic irregularities. 1000×. Photomicrographs by Tillman J. Johnson.

close enough for cross pollination to take place, but this does not usually occur because self-pollination takes place in both species before there would be much probability of cross-pollination.

From the experimental evidence, it is obvious that *S. rotundifolia* and *S.*

virginica are genetically similar enough to hybridize, and a vigorous vegetative hybrid develops; but the genetic dissimilarities between the two parents are great enough to result in a high degree of sterility in the hybrid. While the chromosomes of each of the above species appear to be so much alike in both number and gross morphology, the irregularities in synapsis between the two groups of chromosomes is evidence of qualitative and structural differences between the chromosome groups. Some of the chromosomes of one species have evolved so differently from those of the other species that synapsis no longer occurs between the two or if it does, no chiasmata are formed and the chromosomes separate before first metaphase, becoming univalents. Structural changes must have occurred in some chromosomes because various multiple associations greater than four are present at first metaphase of meiosis in the hybrid (Fig. 2 B). Usually only homologous portions of chromosomes synapse and thus it may be inferred that some chromosomes in the hybrid contain portions which are homologous with portions of two other chromosomes. It may then be further inferred that at some time in the evolutionary history of the parent species segmental translocations between non-homologous chromosomes may have taken place separately within one or both of the species.

An amphiploid is most likely to be fertile and vegetatively vigorous in the first and following generations if its parent species are closely enough related that a vigorous F_1 hybrid results from crosses, yet remotely enough related that the balance between their combined genomes in the F_1 hybrid can not be perpetuated (2). The present hybrid fulfilled both requirements. Therefore attempts were made to create a fertile and vegetatively vigorous amphiploid from the hybrid. Colchicine in water and in a lanolin paste at concentration of .05%, .1%, .2%, .5%, or 1.0% was applied to the growing stem tips of hybrid seedlings. However, all attempts were unsuccessful, as the colchicine only caused a temporary stunting of vegetative growth.

From cytological studies of the hybrid, it appears that if an amphiploid were to be obtained, it might not be entirely fertile during either the first or succeeding generations. Some of the chromosomes of one parent were found to be homologous with those of the other parent, a relation close enough to allow interspecific segregation in their amphiploid. This interspecific segregation might disturb the parental gamete balance and thereby produce sterility or partial sterility in the amphiploid. Furthermore, such an amphiploid would be basically octaploid.

SUMMARY

The diploid chromosome number of *Silene rotundifolia* and *Silene virginica* is reported as 48. They are therefore basically tetraploid. Microsporogenesis is regular in each of these species. Both species also are self- and cross-fertile. F_1 hybrids resulting from crossing them are vegetatively vigorous but highly self and cross sterile. It is suggested that this sterility might be attributed in part or wholly to the observed disturbance of the genetic balance of gametes by meiotic irregularities such as failure of synapsis, formation of multiple associations, chromosome lagging, and the formation of micronuclei.

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